night. The remaining animals including the controls showed loss of activity and were manifestly sick but were lively after 24 hours.

In Series III, guinea pigs were inoculated in a similar manner as in Series II but the recovered filtrate material was inoculated into white mice. In this manner comparatively large doses could be used for a very small animal but the results obtained did not indicate any difference in degree of potency of toxin in the exudate procured from animals with and without phage.

A group of animals, both white mice and guinea pigs, were inoculated with fatal doses of typhoid suspension and another group with phaged doses of typhoid bacilli. In these animals no differences in the period of time of death production was noted. It should be stated that the phaged suspension in these latter experiments was not filtered, whereas, in the other 3 series of experiments the bacteriophage employed was always filtered and tested for sterility before it was employed.

These experiments indicate that the employment of typhoid bacteriophage in conjunction with our previously reported process, has no significant effect upon the potency of toxic substance procured from the typhoid bacillus through this means.

4497

Action of Bacteriophage in Experimental Typhoid Peritonitis.*

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While engaged in certain other experimental work, we had the opportunity to observe the effect of *Bacillus typhosus* bacteriophage upon peritonitis produced in guinea pigs and white mice, by means of the intraperitoneal injection of *Bacillus typhosus*. In view of the apparent possibilities of bacteriophage therapy, it was thought that the observations herein noted in the experimental animals, should be recorded.

A race of bacteriophage virulent for *Bacillus typhosus* was used, which was isolated and kindly furnished us by Professor D'Herelle and which in his hands had been found potent or virulent for several heterologous strains of typhoid bacilli.

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The same strain of *Bacillus typhosus* was used as is described by us in the accompanying article.

At no time were we able to prevent the occurrence of secondary cultures at the end of from 48 hours to 4 or 5 days. A test of the virulence of secondary cultures was made by employing the last tube in a series of 12 dilutions, in which recurrent growth had occurred after lysis had been apparently complete for 5 days. The contents of this tube, at that time, when inoculated intraperitoneally, killed a full grown guinea pig in the same number of hours as did the suspension of the living organisms, used as a control.

To obviate the confusion resulting from the employment of such a phage, all bacteriophage was filtered, tested for sterility and pathogenicity, before inoculation.

The animals used consisted of 36 guinea pigs, and as many white mice.

The first series of experiments were performed to ascertain whether or not the *in vivo* presence of typhosus bacteriophage might prevent the formation of peritonitis. Four guinea pigs were given, intraperitoneally, 2, 3, 4, and 5 cc., respectively, of a salt solution suspension of a young culture of *Bacillus typhosus* and simultaneously there was administered, by the same route, 5 cc. of *Bacillus typhosus* bacteriophage, potency of 10-9, in ordinary bouillon of pH 7.6. A control pig receiving 5 cc. of the culture alone died in 7 hours and another receiving 2 cc. of culture alone died in 24 hours. The treated pigs in this group remained alive and healthy.

We repeated this experiment under as nearly identical circumstances as possible to determine whether this apparent protection was constant. Ten guinea pigs were treated as follows: 6 guinea pigs were inoculated in the same manner as in the first group, with similar controls. In addition 2 pigs were given 5 cc. and 2 cc. of the microorganism plus a simultaneous intraperitoneal injection of ordinary nutrient bouillon, and 2 others, the living microorganism plus 5 cc. of the same culture killed by heat. The apparent protection obtained in the first group was not confirmed, the animals dying the following day and in the same number of hours as did the culture controls, including the 2 animals which received the killed culture of *Bacillus typhosus*. Only the 2 receiving the live microorganisms and simultaneously the nutrient bouillon, remained alive and well.

In the next series of 8 pigs, 4 were injected with 5 cc. (lethal dose) of a salt solution suspension of young living typhoid bacilli intraperitoneally, and 4 with the microorganism plus typhoid bacillus bacteriophage (5 cc.). All of the animals died in approximately

12 hours. The peritoneal exudate present in the peritonitis of the animals that received only the suspension of typhoid bacilli was scanty in amount, highly albuminous, and contained myriads of non-motile bacilli and but few cells; whereas in the pigs inoculated with both bacilli and phage the exudate was copious, serosanguinous in character and contained fewer bacilli but these bacilli were very actively motile.

In order to compare more definitely the relative numbers of living bacteria present in the peritoneal exudate of the animals which had received phage with those inoculated with typhoid culture alone, the respective materials from each group were plated. The pure materials and dilutions of 1-10, 1-100 and 1-1000 were used. A very heavy growth was obtained for all plates but the exudate in which phage had been used showed a distinctly greater number of colonies. It is apparent that many of the bacilli noted by smears and hanging drop in the exudate of the pigs receiving culture alone, were dead. No evidences of lysis or increased phagocytic activity could be detected as the result of the use of bacteriophage.

In another group of animals, the phage was administered an hour before, simultaneously, and an hour following the introduction of the microorganisms. In addition certain animals injected with typhoid bacilli in suspension, were given repeated large doses of bacteriophage at hourly intervals for several hours. The results presented no distinctive differences between the control and treated animals. No evidences of protection or amelioration of the infection were observed in the animals receiving bacteriophage.

Similar experiments were also performed upon white mice. It was thought that since the lethal dose for the animals would approach more closely the direct quantitative relationship to the number of microorganisms susceptible to lysis *in vitro*, greater protection might be procured.

The procedure employed was, in general, the same as that for the guinea pigs with the exception of the smaller dosages. It was found that an injection of 0.1 cc. of the same suspension used for the pigs, proved fatal to the mice in from 7 to 8 hours. The results obtained in the mice were the same as those in the guinea pigs.

In these experiments, it is evident that the employment of *Bacillus typhosus* bacteriophage by this method, played no important rôle in the prophylaxis, production, or therapeusis of experimental typhoid peritonitis in either guinea pigs or white mice.